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Cryptosporidium parvum and *Giardia lamblia* Recovered from Flies on a Cattle Farm and in a Landfill

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Filth flies associated with a cattle barn and a municipal landfill were tested positive by combined immunofluorescent antibody and fluorescent in situ hybridization for *Cryptosporidium parvum* and *Giardia lamblia* on their exoskeletons and in their guts. More pathogens were carried by flies from the cattle barn than from the landfill; 81% of *C. parvum* and 84% of *G. lamblia* pathogens were presumptively viable.

Cryptosporidium parvum and *Giardia lamblia* are zoonotic parasites that cause diarrheal disease worldwide, and *C. parvum* significantly contributes to the mortality of people with impaired immune systems (7, 25). Fluorescent in situ hybridization (FISH) utilizes fluorescently labeled oligonucleotide probes targeted to species-specific sequences of *C. parvum* and *G. lamblia* 18S rRNA (6, 22, 23). Because rRNA has a short half-life and is only present in numerous copies in viable organisms, FISH allows for identification of viable *C. parvum* and *G. lamblia* (6, 17, 23). Synanthropic filth flies are transport hosts for a variety of pathogens of public health importance (14). Fly-associated transmission of *C. parvum* and *G. lamblia* has been discovered relatively recently (2, 5, 8, 11, 12, 13, 15), but it remains unknown if it is a widespread phenomenon and whether these pathogens are capable of initiating human infections.

Nonbiting flies were captured (15) in September at two locations near Gdansk, Poland: (i) dairy cattle farm Bystra (54°18′N, 18°46′E) and (ii) municipal landfill Szadolki (54°19′N, 18°33′E). Flies were killed (11), identified to the family taxon level (15), and preserved in 75% ethanol. Flies were surface eluted (15) and homogenized (10), and the eluant and the homogenate were processed by the cellulose acetate membrane filter dissolution method (9) as described previously (15). Elution ensures recovery of particles from the flys' exoskeleton and homogenization from their guts (15). The resulting pellets were processed by the combined immunofluorescent antibody (IFA) specific to *Cryptosporidium* and *Giardia* spp. and FISH with oligonucleotide probes specific to *C. parvum* and *G. lamblia* as described previously (15). Positive and negative controls were prepared as described previously (15). The numbers of recovered pathogens were adjusted for the efficiency of the cellulose acetate membrane method (78.8%) (9). Statistical analysis was carried out with Statistix 7.0 (Analytical Software, St. Paul, Minn.).

The total numbers of *Muscidae*, *Sarcophagidae*, and *Calliphoridae* flies were 104, 2, and 2, respectively. Only *Muscidae* flies ($n = 100$) were caught at the cattle farm. Combined IFA and FISH analyses clearly differentiated between presumptively viable versus nonviable *C. parvum* oocysts (Fig. 1) and *G. lamblia* cysts (data not shown). Presumptively viable oocysts were intact and revealed a gap between the wall and internal structures, and the sporozoites were visible (Fig. 1). In comparison, dead oocysts, i.e., oocyst shells, frequently had discernible damage to their walls (Fig. 1). Nonviable *G. lamblia* cysts were represented by shells with structurally damaged walls or intact cells with a very small amount of internal structures with diffused appearance. In comparison, presumptively viable cysts were filled out completely with cytoplasm without the gap between the internal structures and the wall. In general, more *C. parvum* than *G. lamblia* was recovered, and this relationship was statistically significant at the cattle farm (rank sum test; $t = 1.22$, $P < 0.05$) (Table 1). Significantly more pathogens per fly were recovered from flies at the cattle farm than from those collected at the landfill (rank sum test; $t = 9.31$, $P < 0.05$) (Table 1). Overall, significantly more presumptively viable than nonviable pathogens were carried by flies (rank sum test; $t = 15.8$, $P < 0.01$) (Table 1). Presumptively viable *C. parvum* oocysts accounted for 81% of all oocysts and over 84% of *G. lamblia* cysts were presumptively viable (Table 1). In general, more *C. parvum* oocysts were found on flys' exoskeletons than in their guts; this relationship was opposite for *G. lamblia* (Table 1).

The present study, together with other studies (2, 5, 8, 13, 15), leaves no doubt that synanthropic flies carry *C. parvum* and *G. lamblia* naturally acquired from unhygienic sites, and it confirms findings that these pathogens are viable (15) and infectious (13) while carried by flies. Thus, nonbiting flies can cause human or animal cryptosporidiosis or giardiasis via deposition of these pathogens on visited surfaces, i.e., food. Food-borne cases and outbreaks of cryptosporidiosis and giardiasis have been extensively documented (1, 4, 24), and therefore it is epidemiologically important to assess the involvement of filth flies in transmission of these food-borne infections.

Mechanical transmission of pathogens by flies is intensive

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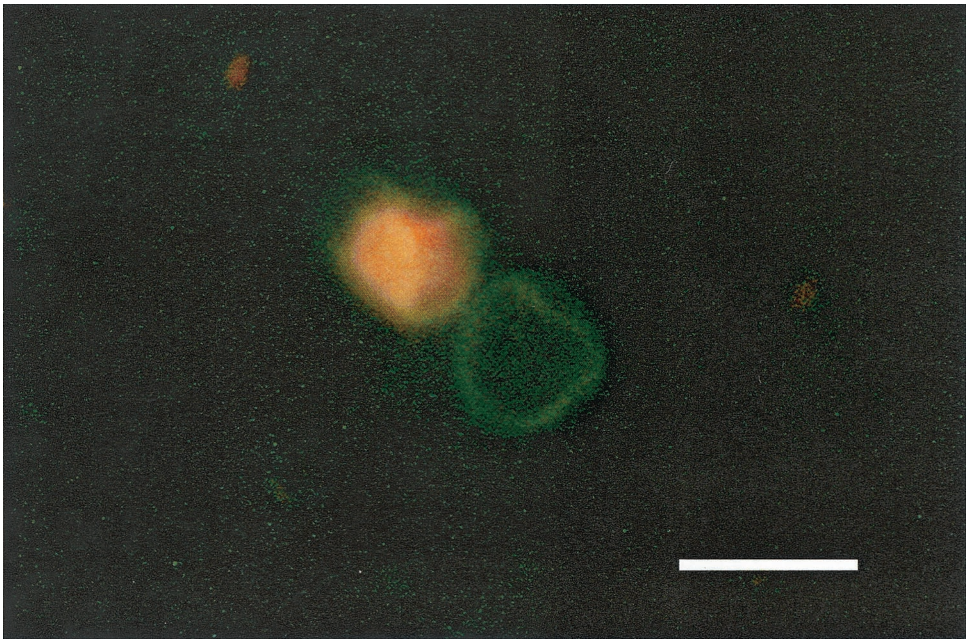

FIG. 1. Combined IFA and FISH image of *C. parvum* oocysts recovered from wild-caught filth flies at dairy cattle barn Bystra, near Gdansk, Poland. Viable *C. parvum* oocyst and oocyst shell, i.e., nonviable oocyst. Scale bar, 5 μm.

because it is achieved through defecation, regurgitation, or mechanical dislodgement (14). Some species of the family *Muscidae* (predominantly collected in the present study) can live up to a month, lay up to six egg batches, and produce up to 12 generations in the summer in temperate climates and several generations during the winter (while indoors) (16). *Muscidae* spp. can travel up to 20 miles (21) and orient their movement toward unsanitary sites (16, 21). The mechanisms of pathogen transmission and the biology and ecology of *Muscidae* spp. (14, 16, 21) indicate a high potential for dissemination of *C. parvum* and *G. lamblia*, which can significantly impact human health, although outbreaks are rarely related to flies. These findings emphasize the need for enforcement of various fly control measures at places such as cattle barns and landfills (16).

The biological basis for more intense pathogen transport in the barn is the fact that cattle feces strongly attracts flies for feeding and breeding (13), and feces viscosity enhances the efficiency of fly hairs and bristles in trapping suspended particles and oocysts (14).

TABLE 1. *C. parvum* oocysts and *G. lamblia* cysts recovered from wild-caught synanthropic flies (Gdansk, Poland) and detected by combined IFA and FISH[a]

Fly collection site	Pathogen extraction site (no. of fly batches)	Mean no. of pathogen cystic stages per SPP.					
		C. parvum			*G. lamblia*		
		Viable	Dead	Overall	Viable	Dead	Overall
1	Exoskeleton (5)	6.3	1.7	8.0	4.8	1.1	5.9
	Gut (5)	9.9	2.0	11.9	1.6	0	1.6
2	Exoskeleton (3)	1.7	0.7	2.4	1.3	0.5	1.8
	Gut (3)	3.5	1.6	5.1	0.5	0.3	0.8
Mean total value		15.7	3.7	19.4	6.1	1.2	7.3

[a] Site 1, dairy cattle farm Bystra; site 2, municipal landfill Szadolki. Flies were separated by family and processed in batches of a maximum of 20 specimens.

FISH has multiple advantages over PCR and IFA for identification of *C. parvum* and *G. lamblia* (3, 6, 15); the most important advantage is assessment of viability of even a single pathogen cell, which can be observed in Fig. 1. Such resolution is not available, or extremely impractical, with any other techniques. For example, with recently developed highly sensitive reverse transcriptase-PCR, the lowest number of *C. parvum* oocysts that can be assessed for viability is 10^3 (18). As only weak autofluorescence of nonstructural debris was observed in the present study, FISH is a suitable technique for identification of *C. parvum* and *G. lamblia* transported not only by flies but also other insect vectors (19, 20, 26).

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REFERENCES

1. **Bean, N. H., S. J. Goulding, C. Lao, and E. J. Lao.** 1996. Surveillance for food borne disease outbreaks—United States, 1988–1992. Morb. Mort. Wkly. Rep. **45:**1–66.
2. **Clavel, A., O. Doiz., S. Morales, M. Varea, C. Seral, F. J. Castillo, J. Fleta, C. Rubio, and R. Gomez-Lus.** 2002. House fly (*Musca domestica*) as a transport vector of *Cryptosporidium parvum*. Folia Parasitol. **49:**163–164.
3. **Deere, D., G. Vesey, M. Milner, K. Williams, N. Ashbolt, and D. A. Veal.** 1998. Rapid method for fluorescent in situ ribosomal RNA labelling of *Cryptosporidium parvum*. J. Appl. Microbiol. **85:**807–818.
4. **Dietz, V., D. Vugis, R. Nelson, J. Wicklund, J. Nadle, K. G. McCombs, and S. Reddy.** 2000. Active, multisite, laboratory-based surveillance for *Cryptosporidium parvum*. Am. J. Trop. Med. Hyg. **62:**368–372.
5. **Doiz, O., A. Clavel, S. Morales, M. Varea, F. J. Castillo, C. Rubio, and R. Gomez-Lus.** 2000. House fly (*Musca domestica*) as a transport vector for *Giardia lamblia*. Folia Parasitol. **47:**330–331.
6. **Dorsch, M. R., and D. A. Veal.** 2001. Oligonucleotide probes for specific

detection of *Giardia lamblia* cysts by fluorescent in situ hybridization. J. Appl. Microbiol. **90:**836–842.

7. **Fayer, R., C. A. Speer, and J. P. Dubey.** 1997. The general biology of *Cryptosporidium*, p. 1–42. *In* R. Fayer (ed.), *Cryptosporidium* and *Cryptosporidiosis*. CRC Press, Boca Raton, Fla.
8. **Follet-Dumoulin, A., K. Guyot, S. Duchatelle, B. Bourel, F. Guilbert, E. Dei-Cas, D. Gosset, and J. C. Cailliez.** 2001. Involvement of insects in the dissemination of *Cryptosporidium* in the environment. J. Eukaryot. Microbiol. **48**(Suppl.)**:**36S.
9. **Graczyk, T. K., M. R. Cranfield, and R. Fayer.** 1997. Recovery of waterborne oocysts of *Cryptosporidium parvum* from water samples by the membrane-filter dissolution method. Parasitol. Res. **83:**121–125.
10. **Graczyk, T. K., R. Fayer, M. R. Cranfield, and D. B. Conn.** 1998. Recovery of waterborne *Cryptosporidium parvum* oocysts by freshwater benthic clams (*Corbicula fluminea*). Appl. Environ. Microbiol. **64:**427–430.
11. **Graczyk, T. K., M. R. Cranfield, R. Fayer, and H. Bixler.** 1999. House flies (*Musca domestica*) as transport hosts of *Cryptosporidium parvum*. Am. J. Trop. Med. Hyg. **61:**500–504.
12. **Graczyk, T. K., R. Fayer, M. R. Cranfield, B. Mhangami-Ruwende, R. Knight, J. M. Trout, and H. Bixler.** 1999. Filth flies are transport hosts of *Cryptosporidium parvum*. Emerg. Infect. Dis. **5:**726–727.
13. **Graczyk, T. K., R. Fayer, R. Knight, B. Mhangami-Ruwende, J. M. Trout, A. J. DaSilva, and N. J. Pieniazek.** 2000. Mechanical transport and transmission of *Cryptosporidium parvum* oocysts by wild filth flies. Am. J. Trop. Med. Hyg. **63:**178–183.
14. **Graczyk, T. K., R. Knight, R. H. Gilman, and M. R. Cranfield.** 2001. The role of non-biting flies in the epidemiology of human infectious diseases. Microbes Infect. **3:**231–235.
15. **Graczyk, T. K., B. H. Grimes, R. Knight, A. J. DaSilva, N. J. Pieniazek, and D. A. Veal.** 2003. Detection of *Cryptosporidium parvum* and *Giardia lamblia* carried by synanthropic flies by combined fluorescent in situ hybridization and monoclonal antibody. Am. J. Trop. Med. Hyg. **68:**228–232.
16. **Greenberg, B.** 1973. Flies and diseases, biology and disease transmission. Princeton University Press, Princeton, N.J.
17. **Jenkins, M., J. M. Trout, J. Higgins, M. Dorsch, D. Veal, and R. Fayer.** 2003. Comparison of tests for viable and infectious *Cryptosporidium parvum* oocysts. Parasitol. Res. **89:**1–5.
18. **Jenkins, M. C., J. Trout, M. S. Abrahamsen, J. Higgins, and R. Fayer.** 2000. Estimating viability of *Cryptosporidium parvum* oocysts using reverse transcriptase-polymerase chain reaction (RT-PCR) directed at mRNA encoding amyloglucosidase. J. Microbiol. Methods **34:**97–106.
19. **Kasprzak, W., and A. Majewska.** 1981. Transmission of *Giardia* cysts. I. Role of flies and cockroaches. Wiad. Parazytol. **27:**555–563.
20. **Mathison, B. A., and O. Ditrich.** 1999. The fate of *Cryptosporidium parvum* oocysts ingested by dung beetles and their possible role in the dissemination of cryptosporidiosis. J. Parasitol. **85:**678–681.
21. **Murvosh, C. M., and C. W. Thaggard.** 1966. Ecological studies of the house fly. Ann. Entomol. Soc. Am. **59:**533–547.
22. **Vesey, G., N. Ashbolt, G. Wallner, M. Dorsch, K. L. Williams, and D. A. Veal.** 1995. Assessing *Cryptosporidium parvum* oocyst viability with fluorescent in-situ hybridization using ribosomal RNA probes and flow cytometry, p. 133–138. *In* W. B. Betts, D. Casemore, C. Fricker, H. Smith, and J. Watkins (ed.), Protozoan parasites and water. Royal Society of Chemistry, Cambridge, United Kingdom.
23. **Vesey, G., N. Ashbolt, E. J. Fricker, D. Deere, K. L. William, D. A. Veal, and M. Dorsch.** 1998. The use of a ribosomal RNA targeted oligonucleotide probe for fluorescent labelling of viable *Cryptosporidium parvum* oocysts. J. Appl. Microbiol. **85:**429–440.
24. **Wallace, D. J., T. VanGilder, S. Shalow, T. Fiorentino, S. D. Segler, K. E. Smith, B. Shiferar, R. Etzel, W. E. Garthright, and F. J. Angulo.** 2000. Incidence of foodborne illness reported by the foodborne diseases active surveillance network (FoodNet)-1997. J. Food Prot. **63:**807–809.
25. **Wolfe, M. S.** 1992. Giardiasis. Clin. Microbiol. Rev. **5:**93–100.
26. **Zerpa, R., and L. Huicho.** 1994. Childhood cryptosporidial diarrhea associated with identification of *Cryptosporidium* sp. in the cockroach, *Periplaneta americana*. Pediatr. Infect. Dis. **13:**546–548.